

# **DNA Camouflage**

## **Supplementary Information**

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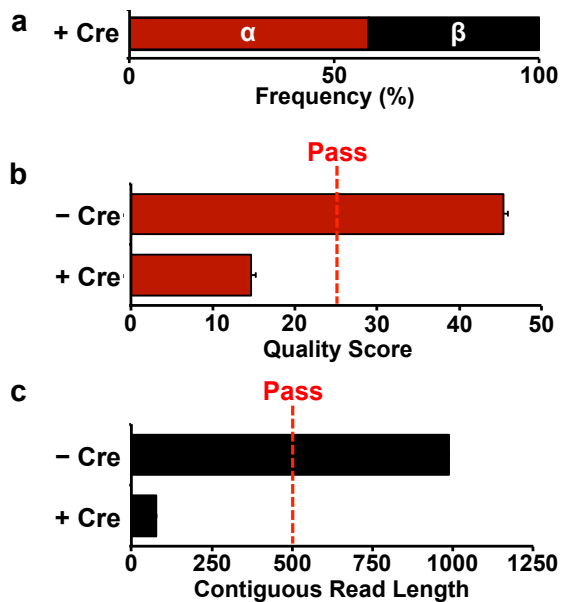
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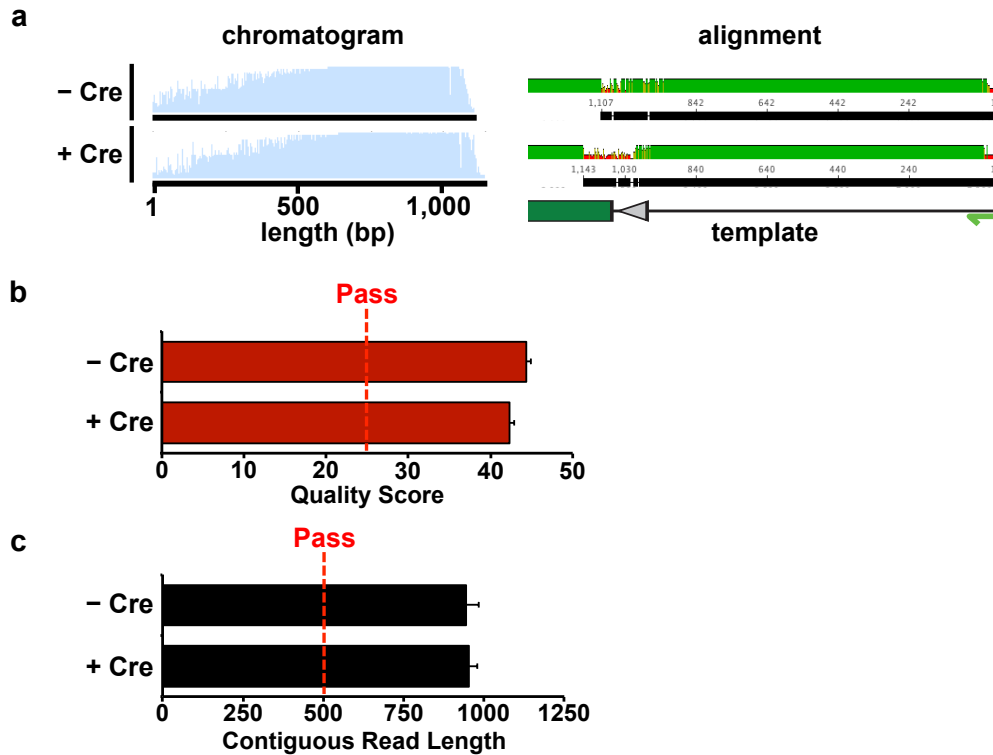
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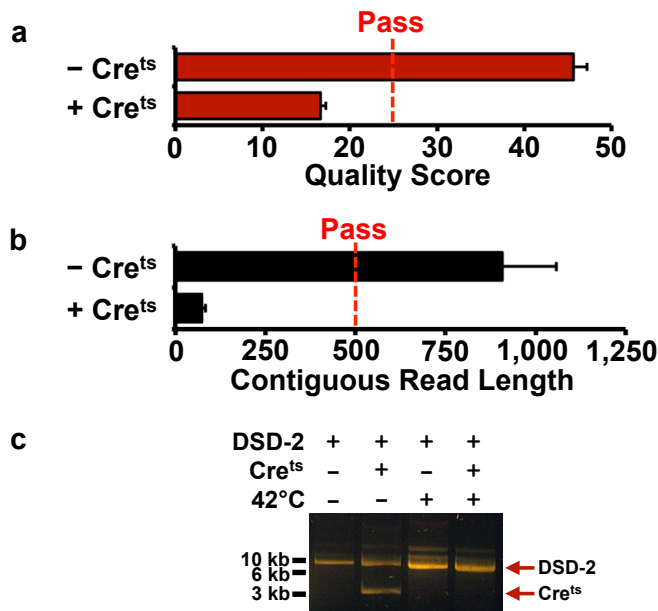
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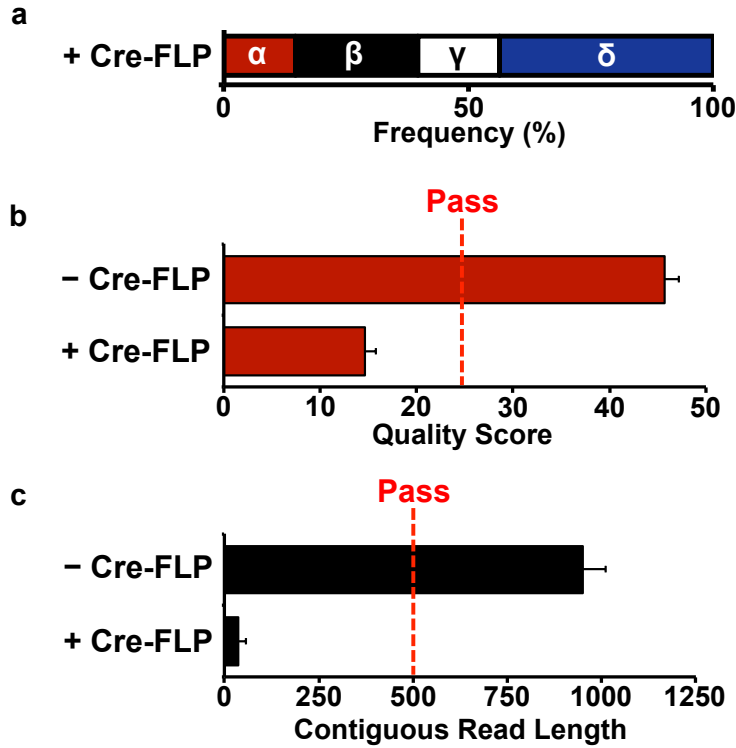
**Supplementary Figure 1** DNA camouflage with the 2-state device. **(a)** In the presence of Cre, DSD-2[ $\alpha$ ] was randomly shuffled between  $\alpha$  and  $\beta$  states within a cellular population. **(b)** Quality score (QS) values of sequencing reactions of DSD-2[ $\alpha$ ] maintained in the absence and presence of Cre. **(c)** Contiguous read length (CRL) scores of sequencing reactions of DSD-2[ $\alpha$ ] maintained in the absence and presence of Cre. All experiments were performed in triplicate, error bars represent  $\pm 1$  standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.



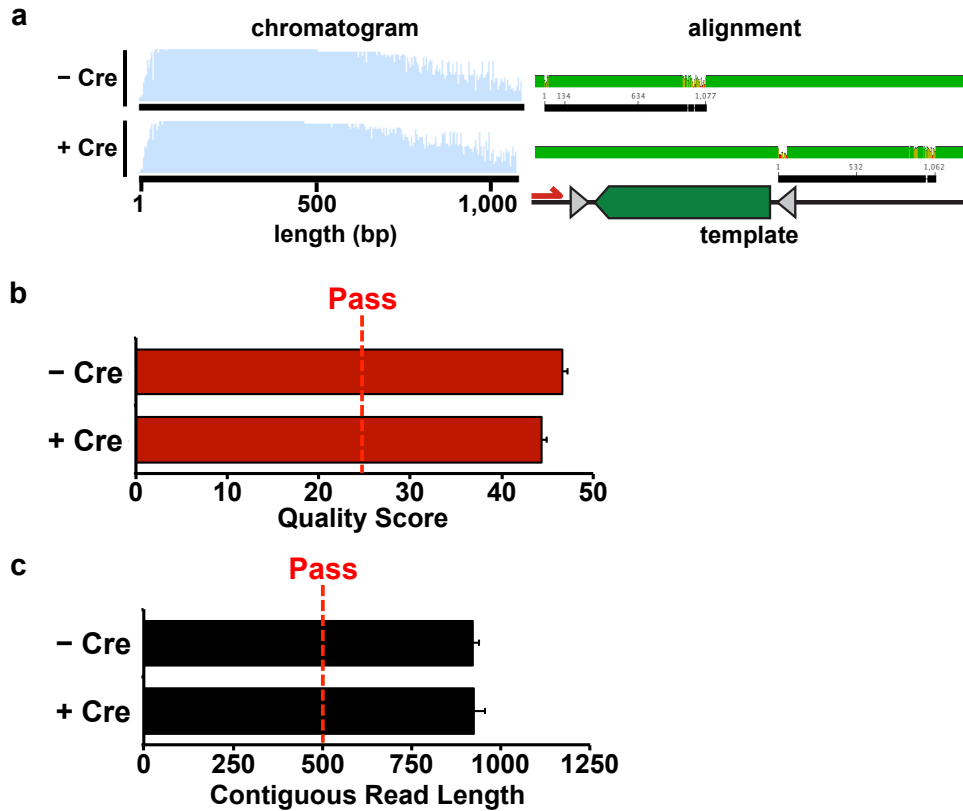
**Supplementary Figure 2** DNA shuffling does not comprise sequencing outside of DSDs. **(a)** Sequencing of 1 kb downstream of DSD-2[ $\alpha$ ] produces high quality sequencing reads that align with the template in the absence and presence of Cre. **(b)** Quality score (QS) and **(c)** Contiguous read length (CRL) scores of sequencing reactions shown in **a**. All experiments were performed in triplicate, error bars represent  $\pm 1$  standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.



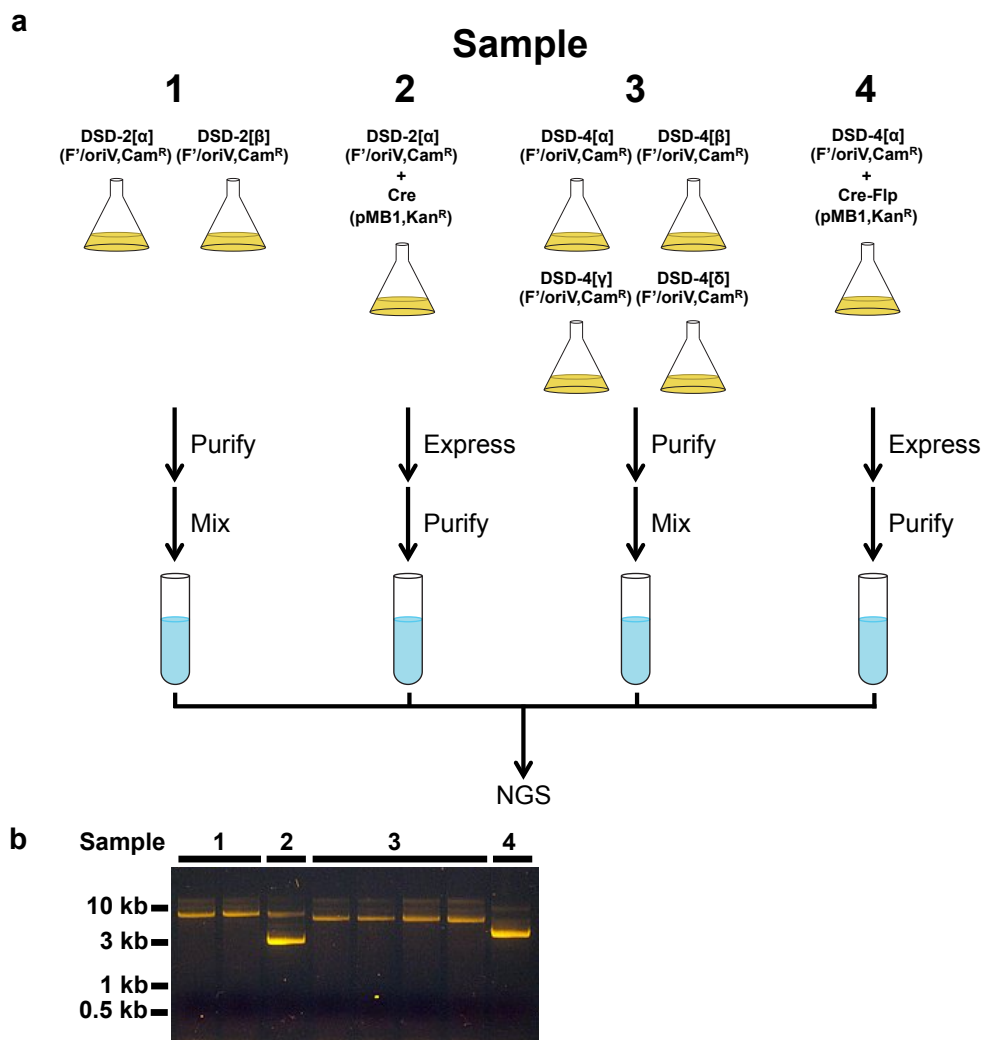
**Supplementary Figure 3** DNA camouflage with a switchable 2-state device. **(a)** Quality score (QS) and **(b)** Contiguous read length (CRL) scores of sequencing reactions of DSD-2[α] maintained in the absence and presence of Cre<sup>ts</sup>. **(c)** The plasmid encoding Cre<sup>ts</sup> can be cured out of cells by growing cells at 42°C. All experiments were performed in triplicate, error bars represent  $\pm 1$  standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.



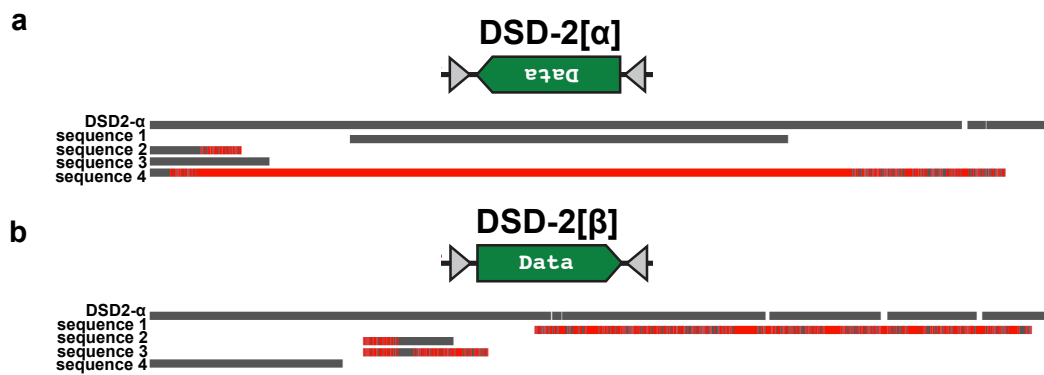
**Supplementary Figure 4** DNA camouflage with the 4-state device. **(a)** In the presence of Cre and Flp, DSD-4[ $\alpha$ ] was randomly shuffled between  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  states within a cellular population. **(b)** Quality score (QS) values of sequencing reactions of DSD-4[ $\alpha$ ] maintained in the absence and presence of Cre and Flp. **(c)** Contiguous read length (CRL) scores of sequencing reactions of DSD-4[ $\alpha$ ] maintained in the absence and presence of Cre and Flp. All experiments were performed in triplicate, error bars represent  $\pm 1$  standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.



**Supplementary Figure 5** Shuffling of DSD-2[ $\alpha$ ]<sup>p15A</sup> leads to data excision. **(a)** When DSD-2[ $\alpha$ ] is placed on a multi-copy plasmid containing a p15A origin (DSD-2[ $\alpha$ ]<sup>p15A</sup>), data is maintained in the absence of Cre but excised in the presence of Cre. **(b)** Quality score (QS) and **(c)** Contiguous read length (CRL) scores for sequence reactions shown in **a**. All experiments were performed in triplicate, error bars represent  $\pm 1$  standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.

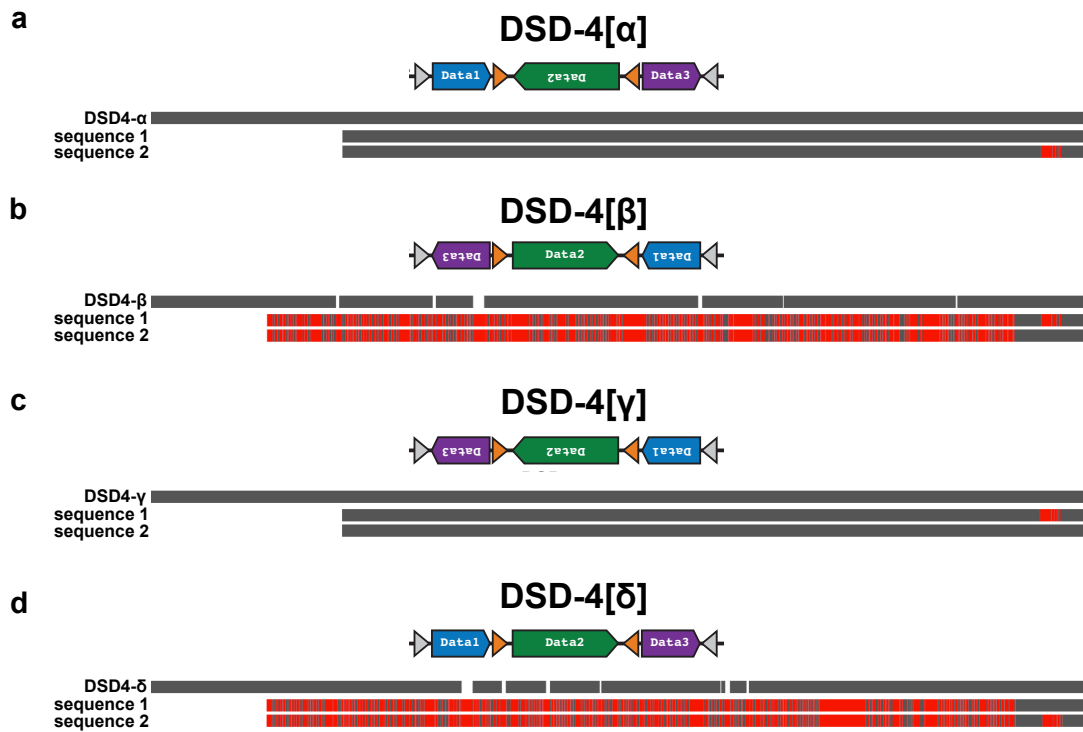


**Supplementary Figure 6** Next-generation sequencing (NGS) of 2-state and 4-state devices. **(a)** Samples 1 and 3: DSD-2[ $\alpha/\beta$ ] and DSD4-[ $\alpha/\beta/\gamma/\delta$ ] were each separately prepared, purified, and mixed at equal concentration in dH<sub>2</sub>O. Sample 2 and 4: DSD-2[ $\alpha$ ] and DSD-4[ $\alpha$ ] were shuffled with Cre and Cre-Flp recombinases respectively, and then purified, and stored in dH<sub>2</sub>O. **(b)** Samples from **(a)** run on an agarose gel to demonstrate the purity.

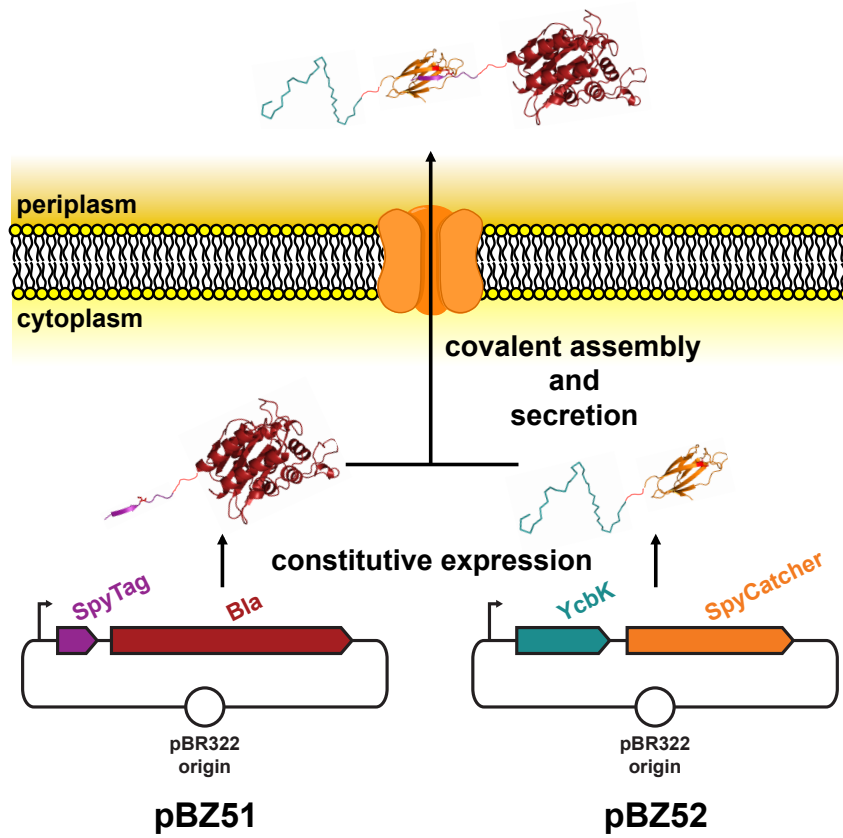


**Supplementary Figure 7** NGS identified sequences for Sample 1. Sequences identified by the outside party for Sample 1 (**Supplementary Table 4**) aligned against (a) DSD-2[ $\alpha$ ] and (b) DSD-2[ $\beta$ ] templates. Gray bars represent areas of perfect sequence alignment and red bars represent areas of sequence misalignment.

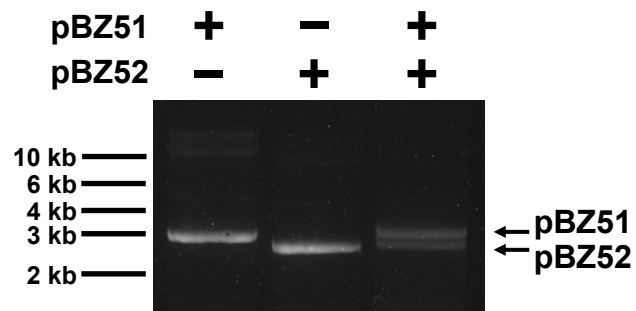




**Supplementary Figure 8** NGS identified sequences for Sample 3. Sequences identified by the outside party for Sample 3 (**Supplementary Table 4**) aligned against (a) DSD-4[α], (b) DSD-4[β], (c) DSD4-[γ], and (d) DSD4-[δ] templates. Gray bars represent areas of perfect sequence alignment and red bars represent areas of sequence misalignment.



**Supplementary Figure 9** Schematic of the addiction module.



**Supplementary Figure 10** pBZ51 and pBZ52 are stably maintained in *E. coli*. Cells transformed with pBZ51 (selected on Kan), pBZ52 (selected on Kan), and pBZ51 + pBZ52 (selected on Amp) were grown overnight, and plasmid DNA was extracted and run on a 1% agarose gel. Cells co-transformed with pBZ51 and pBZ52 were able to stably maintain both plasmids under Amp selection.

Sample	1	2	3	4
Total Sequences	2,035,696	2,827,422	3,762,818	2,665,635
% GC	48	49	47	46

**Supplementary Table 1** NGS analysis of samples 1-4. Over 2 million ~300 bp reads were produced from NGS sequencing of samples 1-4 (**Supplementary Fig. 6**), with GC contents similar to expected values. DSD-2[ $\alpha/\beta$ ]: 9,549 bp/47.8% GC, Cre: 4,452 bp/49.8% GC, DSD4-[ $\alpha/\beta/\gamma/\delta$ ]: 8,204 bp/46.8% GC, Cre-Flp: 5,769 bp/46.9% GC.

<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>Sequence size</b>	4,484,782	109,143	4,575,261	238,314
<b>Number of scaffolds</b>	711	248	500	536
<b>% GC</b>	50.7	49.3	50.7	50.1
<b>Shortest contig size</b>	301	300	306	300
<b>Median sequence size</b>	3,897	360	3,943	390
<b>Mean sequence size</b>	6,307.7	440.1	9,150.5	444.6
<b>Longest contig size</b>	51,023	5,385	93,737	5,397
<b>Number of subsystems</b>	564	2	576	2
<b>Number of coding sequences</b>	4,300	64	4,410	190
<b>Number of RNAs</b>	34	0	30	0

**Supplementary Table 2** Assembly of NGS reads from samples 1-4. Here, the statistics of the assembled scaffolds from are shown.

Sample	Total Scaffolds	Aligned Scaffolds	% Aligned	Identified Vectors
1	711	12	1.7	<ul style="list-style-type: none"> <li>• pBluescriptR (Amp<sup>R</sup>)</li> <li>• pDONR221 (Kan<sup>R</sup>)</li> <li>• pOTB7 (Cam<sup>R</sup>)</li> </ul>
2	248	3	1.2	<ul style="list-style-type: none"> <li>• pBluescriptR (Amp<sup>R</sup>)</li> <li>• pDONR221 (Kan<sup>R</sup>)</li> <li>• pOTB7 (Cam<sup>R</sup>)</li> </ul>
3	500	10	2.0	<ul style="list-style-type: none"> <li>• pBluescriptR (Amp<sup>R</sup>)</li> <li>• pDONR221 (Kan<sup>R</sup>)</li> <li>• pOTB7 (Cam<sup>R</sup>)</li> </ul>
4	536	6	1.1	<ul style="list-style-type: none"> <li>• pBluescriptR (Amp<sup>R</sup>)</li> <li>• pDONR221 (Kan<sup>R</sup>)</li> <li>• pOTB7 (Cam<sup>R</sup>)</li> <li>• pK7-GFP (Amp<sup>R</sup>)</li> </ul>

**Supplementary Table 3** Identification of annotated and assembled samples 1-4. Since there was no prior information provided regarding samples 1-4, the assembled scaffolds (**Supplementary Table 2**) were blasted against a plasmid database (<http://plasmid.med.harvard.edu/>) by the outside party. Identified hits were based on >90% sequence identity and a minimum of 100 bp alignment length.

Sample	Sequence Number	Identified Insert
1	1	TTCATCCATGCCATGTGTAATCCAGCAGCTGTTACAAACTCAAGAAGGACCATTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGGACAGGTAATGGTTGTCTGGTAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTGTCTAATTTGAAGTTAACTTTGATTCATCTTTTGTGCTGCCATGATGATACATTGTG TGAGTTATAGTTGTAATCCAAATTTGTGTCGAAGATGTTCCATCTCTTTTAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTCTTGTAGTCCCGTCATCTTTGAAAAATATAGTTCTTCTGTACATAACCTTCGG GCATGGCACTCTTGAAGAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCAGTAGTGCAAAATAATTAAGGTAAGTTTCCGTATGTGCATCACCTTCAACCTCTCCA CTGACAGAAAAATTTGCCCCATTAACATCACCATCTAATTCACAAGAATTGGGACAACTCCAGTGAAAAAGTTCTTCTCTTTACGCA TGGTATATCTCCTCTTAAAGTGGTCAGTGCCTCTGCTGATGTGCTCAGTATCTTGTATCCGCTCACAATGTAAATTTG
	2	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAGCGGAT ACAATTGTGAGCGGATAACAATTTACATTGTGAGCGGATAACAAGTACTGAGCACATCAGCAGGACGCATGACC
	3	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAGCGGAT ACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTCCCGGCACATTTCCCGGAAAGTGCCACCTAGGTATCTGGCACTACG TTCAGTAACTGAAGCTCGAATCCAGTACTCGACGTC
	4	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCTAGCTATGAGAAACAGTAGAGTTGCGATAAAAAGCGTCAGGTAGGATCC GCTAATCTTATGGATAAAAAATGCTATGGCATAGCAAAAGTGTGACGCCGTGCAAAATCAATGTGGACTTTTCTCGCGGTGATATAGA CACTTTTGTACGGTTTTTGTGATGCTTTGGTCCCGCTTTGTACAGAATGCTTTAATAAGCGGGTTACCGTTTTGGTTAGCGA GAAGAGCCAGTAAAGAGCGCATGACGGCAATGTCTGATGCAATATGGACAATTGGTTCTTCTGCTGCTGATTATAGTCTGGG
2	no insert sequence identified	
3	1	TTCATCCATGCCATGTGTAATCCAGCAGCTGTTACAAACTCAAGAAGGACCATTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGGACAGGTAATGGTTGTCTGGTAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTGTCTAATTTGAAGTTAACTTTGATTCATCTTTTGTGCTGCCATGATGATACATTGTG TGAGTTATAGTTGTAATCCAAATTTGTGTCGAAGATGTTCCATCTCTTTTAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTCTTGTAGTCCCGTCATCTTTGAAAAATATAGTTCTTCTGTACATAACCTTCGG GCATGGCACTCTTGAAGAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCAGTAGTGCAAAATAATTAAGGTAAGTTTCCGTATGTGCATCACCTTCAACCTCTCCA CTGACAGAAAAATTTGCCCCATTAACATCACCATCTAATTCACAAGAATTGGGACAACTCCAGTGAAAAAGTTCTTCTCTTTACGCA TGGTATATCTCCTCTTAAAGTGGTCAGTGCCTCTGCTGATGTGCTCAGTATCTTGTATCCGCTCACAATGTAAATTTGTTATCCGC TCACAATTGTATCCGCTCATGAATTAATCTTAGAAGTCTCTATATCTTCTAGAGAATAGGAACCTCAGGCATGTGATGAATCGTAGT CTCAATAACTTCGTATAGCATACATTATACGAAGTTAT
	2	TTCATCCATGCCATGTGTAATCCAGCAGCTGTTACAAACTCAAGAAGGACCATTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGGACAGGTAATGGTTGTCTGGTAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTGTCTAATTTGAAGTTAACTTTGATTCATCTTTTGTGCTGCCATGATGATACATTGTG TGAGTTATAGTTGTAATCCAAATTTGTGTCGAAGATGTTCCATCTCTTTTAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTCTTGTAGTCCCGTCATCTTTGAAAAATATAGTTCTTCTGTACATAACCTTCGG GCATGGCACTCTTGAAGAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCAGTAGTGCAAAATAATTAAGGTAAGTTTCCGTATGTGCATCACCTTCAACCTCTCCA CTGACAGAAAAATTTGCCCCATTAACATCACCATCTAATTCACAAGAATTGGGACAACTCCAGTGAAAAAGTTCTTCTCTTTACGCA TGGTATATCTCCTCTTAAAGTGGTCAGTGCCTCTGCTGATGTGCTCAGTATCTTGTATCCGCTCACAATGTAAATTTGTTATCCGC TCACAATTGTATCCGCTCATGAATTAATCTTAGAAGTCTCTATATCTTCTAGAGAATAGGAACCTCCTCATCGAGCATCAATGAAA CTGCATAACTTCGTATAGCATACATTATACGAAGTTAT
4	no insert sequence identified	

**Supplementary Table 4** Identified sequences by the outside party following NGS analysis and sequence assembly. These sequences were assembled once the sequence of the backbone vectors were provided to the outside party.

Construct	Plasmid Name	Plasmid Backbone	Sequence	Legend
Cre	pBZ14	pET28a (pBR322 origin and Kan <sup>R</sup> only)	<p>TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA  CATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATT  TACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGA  TGAGGTTTCGCAAGAACCTGATGGACATGTTCAAGGATCGCCAGGCGTTTCT  GAGCATACCTGGAAAATGCTTCTGTCCGTTTCCCGGTCGTGGCGCGCATGGT  GCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCCGCA  TTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAATATCCAGCAAC  ATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGCTGCCACGACCAAG  TGACAGCAATGCTGTTCACTGGTTATGCGGCGGATCCGAAAAAGAAACGTT  GATGCCGTTGAACGTGCAAAACAGGCTCTAGCGTTTCAACGCACTGATTTCG  ACCAAGTTCTGTTCACTCATGAAAATAGCGATCGTCCAGGATATACGTAA  TCTGGCATTCTGGGGATTGCTTATAACACCTGTTACGTATAGCCGAAATTG  CCAGGATCAGGGTTAAAGATATCTACGTACTGACGGTGGGAGAATGTTAAT  CCATATTGGCAGAACGAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGC  ACTTAGCCTGGGGTAACTAACTGGTCGAGCGATGGATTTCCTGCTCTGGT  GTAGCTGATGATCCGAATAACTACCTGTTTTCGGGGTCAGAAAAATGGTGT  TGCCGCGCATCTGCCACGACGCTATCAACTCGCGCCTGGAAGGAT  TTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGA  GATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCCGAGCCGCGCAGATA  TGGCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGCTGGA  CCAATGTAATATTGTATGAACATATCCGTAACCTGGATAGTGAACAGGG  GCAATGGTGGCCTGCTGGAAGATGGCGATTAAAGTCGACAACCTAGGAAAAA  CCTGAGGAAAAATGCATAGCTAGAGGCATCAATAAAACGAAAGGCTCAGTCG  AAAGACTGGGCTTTTCGTTTATCTGTTGTTGTGCGTGAACGCTCTCCTGAG  TAGGACAAATCCGCCGGGAGCGGATTGAACGCTGCGAAGCAACGGCCGG  AGGGTGGCGGGCAGGACGCCGCCATAAAGTCCAGGCATCAAAATTAAGCA  GAAGGCCATCCTGACGGATGGCCTTTTTCGCTTCTACAAA</p>	<p>P<sup>LtetO-1</sup>  Cre  Terminator  Spacer</p>
Cre <sup>ts</sup>	pBZ20	pKD46 (origin and Amp <sup>R</sup> only)	<p>TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA  CATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATT  TACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGA  TGAGGTTTCGCAAGAACCTGATGGACATGTTCAAGGATCGCCAGGCGTTTCT  GAGCATACCTGGAAAATGCTTCTGTCCGTTTCCCGGTCGTGGCGCGCATGGT  GCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCCGCA  TTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAATATCCAGCAAC  ATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGCTGCCACGACCAAG  TGACAGCAATGCTGTTCACTGGTTATGCGGCGGATCCGAAAAAGAAACGTT  GATGCCGTTGAACGTGCAAAACAGGCTCTAGCGTTTCAACGCACTGATTTCG  ACCAAGTTCTGTTCACTCATGAAAATAGCGATCGTCCAGGATATACGTAA  TCTGGCATTCTGGGGATTGCTTATAACACCTGTTACGTATAGCCGAAATTG  CCAGGATCAGGGTTAAAGATATCTACGTACTGACGGTGGGAGAATGTTAAT  CCATATTGGCAGAACGAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGC  ACTTAGCCTGGGGTAACTAACTGGTCGAGCGATGGATTTCCTGCTCTGGT  GTAGCTGATGATCCGAATAACTACCTGTTTTCGGGGTCAGAAAAATGGTGT  TGCCGCGCATCTGCCACGACGCTATCAACTCGCGCCTGGAAGGAT  TTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGA  GATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCCGAGCCGCGCAGATA  TGGCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGCTGGA  CCAATGTAATATTGTATGAACATATCCGTAACCTGGATAGTGAACAGGG  GCAATGGTGGCCTGCTGGAAGATGGCGATTAAAGTCGACAACCTAGGAAAAA  CCTGAGGAAAAATGCATAGCTAGAGGCATCAATAAAACGAAAGGCTCAGTCG  AAAGACTGGGCTTTTCGTTTATCTGTTGTTGTGCGTGAACGCTCTCCTGAG  TAGGACAAATCCGCCGGGAGCGGATTGAACGCTGCGAAGCAACGGCCGG  AGGGTGGCGGGCAGGACGCCGCCATAAAGTCCAGGCATCAAAATTAAGCA  GAAGGCCATCCTGACGGATGGCCTTTTTCGCTTCTACAAA</p>	<p>P<sup>LtetO-1</sup>  Cre  Terminator  Spacer</p>
Cre-Flp	pBZ17	pET28a (pBR322 origin and Kan <sup>R</sup> only)	<p>TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA  CATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATT  TACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGA  TGAGGTTTCGCAAGAACCTGATGGACATGTTCAAGGATCGCCAGGCGTTTCT  GAGCATACCTGGAAAATGCTTCTGTCCGTTTCCCGGTCGTGGCGCGCATGGT  GCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCCGCA  TTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAATATCCAGCAAC  ATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGCTGCCACGACCAAG  TGACAGCAATGCTGTTCACTGGTTATGCGGCGGATCCGAAAAAGAAACGTT  GATGCCGTTGAACGTGCAAAACAGGCTCTAGCGTTTCAACGCACTGATTTCG  ACCAAGTTCTGTTCACTCATGAAAATAGCGATCGTCCAGGATATACGTAA  TCTGGCATTCTGGGGATTGCTTATAACACCTGTTACGTATAGCCGAAATTG  CCAGGATCAGGGTTAAAGATATCTACGTACTGACGGTGGGAGAATGTTAAT  CCATATTGGCAGAACGAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGC  ACTTAGCCTGGGGTAACTAACTGGTCGAGCGATGGATTTCCTGCTCTGGT  GTAGCTGATGATCCGAATAACTACCTGTTTTCGGGGTCAGAAAAATGGTGT  TGCCGCGCATCTGCCACGACGCTATCAACTCGCGCCTGGAAGGAT  TTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGA  GATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCCGAGCCGCGCAGATA  TGGCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGCTGGA  CCAATGTAATATTGTATGAACATATCCGTAACCTGGATAGTGAACAGGG  GCAATGGTGGCCTGCTGGAAGATGGCGATTAAAGTCGACAACCTAGGAAAAA  AATTTTGTAACTTTAAGAAGGAGATATACCATGAGCCAAATTGATATATTAT  GTAAACACCACTAAGGTCTGTTTCGTGAGTTTGTGAAAGGTTTGAAG  ACCTTCAGGGGAAAAATAGCATCATGTGCTGCTGAACCTAATTTATGTT  GGATGATTACTCATAACGGAACAGCAATCAAGAGAGCCACATTTCAGGCTAT  AATACTATCATAAGCAATTCGCTGAGTTTCGATATTGTCAACAACTCACTCCAG  TTTAAATACAGAGCGCAAAAGCAACAATTCTGGAAGCCTCATTAAGAAAT  AATTCCTGCTTGGGAATTTACAATTATCCTTACAATGGACAAAAACATCAATC  TGATATCACTGATATTGAAGTAGTTTGAATACAGTTTCAATCATCGGAAG</p>	<p>P<sup>LtetO-1</sup>  Cre  Flp  Terminator  Spacer</p>



			AAGCAGATAAGGGAATAGCCACAGTAAAAAATGCTTAAAGCACTTCTAAGT GAGGGTGAAGGCATCTGGGAGATCACTGAGAAAACTAAATTCGTTTGAGT ATACCTCGAGATTTACAAAAACAAAACTTTATACCAATTCCTCTCTAGCTA CTTTCATCAATTGTGGAAGATTGAGCGATATTAAGAACGTTGATCCGAAATCA TTTAAATTAGTCCAAATAAGTATCTGGGAGTAATAATCCAGTGTTTGTAGACA GAGACAAAGACAAGCGTTAGTAGGCACATATACCTCTTTAGCGCAAGGGGTA GGATCGATCCACTTGTATATTTGGATGAATTTTGGAGAACTCTGAACCAGTC CTAAACGAGTAAATAGGACCGGCAATTCTTCAAGCAACAAACAGGAATACCA ATTATTAAGATAACTTAGTCAGATCTGACAAACAGGCTTTGAAGAAAAATG CGCCTTATCCAATCTTTGCTATAAAGAATGGCCAAAATCTCACATTGGAAGA CATTTGATGACCTCATTCTGTCAATGAAGGGCCTAACGGAGTTGACTAATGT TGTGGGAAATTGGAGCGATAAGCGTGCTTCTGCCGTGGCCAGGACAAGTAT ACTCATCAGATAACAGCAATACCTGATCACTACTTCGCACTAGTTTCTCGGTA CTATGCATATGATCCAATATCAAGGAAATGATAGCAATTGAAGGATGAGACTA ATCCAATTGAGGAGTGGCAGCATATAGAAGCTAAAGGGTAGTGCTGAAGG AAGCATACGATACCCCGCATGGAATGGGATAATATCACAGGAGGACTAGAC TACCTTTTACCTCTACATAAATTAATAGTCGACAACCTAGGAAAAACCTGAGG AAAAATGCATAGCTAGAGGCATCAATAAAACGAAGGCTCAGTCAAGAGCT GGGCCTTTCGTTTATCTGTTGTTTGTGGTGAACGCTCTCCTGAGTAGGACA AATCGCGCGGAGCGGATTTGAACGCTGCGAAGCAACGGCCGAGGCTGT GCGGGCAGGACGCCGCCATAAATCTGCCAGGCATCAATTAAGCAGAAGGC CATCTGACGGATGGCCTTTTGTGCTTTCTACAAA	
DSD-2[α]	pBZ22	pBAC- LacZ (F'/ori <sup>V</sup> origins and Cam <sup>R</sup> )	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTGATGCTCG ATGAGTTTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGATTTAG AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGGAAAAGTGCCACCTA GGTATCTGGCACTACGTTTCAGGTAACCTGAAGCTCGAATCCAGTACTCGAGC TCTCTAGGGCGGCGGATTTGTCTACTCAGGAGAGCGTTACCCGACAAAGCA CAGATAAAACGAAAGGCCAGCTTTTCGACTGAGCCTTTTCGTTTATTTGATG CCTCTAGCAGCGGTACCTGGTGGCGCGCCTTATTTGTATAGTTCATCCATGC CATGTGTAATCCAGCAGCTGTTACAACTCAAGAAGGACCATGTGTCCTCT CTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTG TCTGGTAAAAGGACAGGGCCATCGCCCAATTGGAGTATTTTGTGATATGGTC TGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAECTT GATTCATTCTTTTGTGTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTT GTATTCGAATTTGTGTCCAAGAAATGTTTCCATCTTCTTAAATCAATACCTTTT AACTCGATTCTATTAACAAGGGTATCACCTTCAAACCTTGACTTCAGCAGCTGT CTTGATGTTCCCGTCATCTTTGAAAAATATAGTCTTCTCCTGTACATAACCTTC GGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCG CAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTGGCCATGGAAC AGGTAGTTTTCCAGTAGTGCAAAATAAATTTAAGGGTAAGTTTTCCGTATGTTG CATCACCTTACCCTCTCCACTGACAGAAAATTTGTGCCATTAACATCACCA TCTAATTCAACAAGAATTGGGACAACCTCCAGTAAAAAGTTCTTCTCCTTACG CATGGTATATCTCCTTCTAAAGTGGTCAGTGCGTCTGCTGATGTGCTCAGT ATCTTGTTATCCGCTCACAATGTAATTTGTTATCCGCTCACAATTTGATCCGCT CATGAATTAATTTAGGCATATTCAAATCGTTTTCGTTACCGCTTCAGAGCAT CATGACAGAACTACTTCTCTATAAACGCTACACAGGCTCCTGAGATTATAAA TGCGGATCTGTCCAGACTAATAATCAGACCGACGAAGAAACCAATTGTCCA TATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAAC CAAACCGGTAACCCCGCTTATTAAGCAATTCGTAAACAAGCGGGACCAAA GCCATGACAAAAACCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCC ACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTATCCA TAAGATTAGCGGATCTTACCTGACGCTTTTATCGCAACTCTCTACTGTTTCT CCATAGTAGCATAACTTCGTATAGCATACATTATACGAAGTTAT	loxP Data
DSD-2[α] <sup>p15A</sup>	pBZ19	p15A (origin and Cam <sup>R</sup> only)	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTGATGCTCG ATGAGTTTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGATTTAG AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGGAAAAGTGCCACCTA GGTATCTGGCACTACGTTTCAGGTAACCTGAAGCTCGAATCCAGTACTCGAGC TCTCTAGGGCGGCGGATTTGTCTACTCAGGAGAGCGTTACCCGACAAAGCA CAGATAAAACGAAAGGCCAGCTTTTCGACTGAGCCTTTTCGTTTATTTGATG CCTCTAGCAGCGGTACCTGGTGGCGCGCCTTATTTGTATAGTTCATCCATGC CATGTGTAATCCAGCAGCTGTTACAACTCAAGAAGGACCATGTGTCCTCT CTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTG TCTGGTAAAAGGACAGGGCCATCGCCCAATTGGAGTATTTTGTGATATGGTC TGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAECTT GATTCATTCTTTTGTGTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTT GTATTCGAATTTGTGTCCAAGAAATGTTTCCATCTTCTTAAATCAATACCTTTT AACTCGATTCTATTAACAAGGGTATCACCTTCAAACCTTGACTTCAGCAGCTGT CTTGATGTTCCCGTCATCTTTGAAAAATATAGTCTTCTCCTGTACATAACCTTC GGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCG CAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTGGCCATGGAAC AGGTAGTTTTCCAGTAGTGCAAAATAAATTTAAGGGTAAGTTTTCCGTATGTTG CATCACCTTACCCTCTCCACTGACAGAAAATTTGTGCCATTAACATCACCA TCTAATTCAACAAGAATTGGGACAACCTCCAGTAAAAAGTTCTTCTCCTTACG CATGGTATATCTCCTTCTAAAGTGGTCAGTGCGTCTGCTGATGTGCTCAGT ATCTTGTTATCCGCTCACAATGTAATTTGTTATCCGCTCACAATTTGATCCGCT CATGAATTAATTTAGGCATATTCAAATCGTTTTCGTTACCGCTTCAGAGCAT CATGACAGAACTACTTCTCTATAAACGCTACACAGGCTCCTGAGATTATAAA TGCGGATCTGTCCAGACTAATAATCAGACCGACGAAGAAACCAATTGTCCA TATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAAC CAAACCGGTAACCCCGCTTATTAAGCAATTCGTAAACAAGCGGGACCAAA GCCATGACAAAAACCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCC ACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTATCCA TAAGATTAGCGGATCTTACCTGACGCTTTTATCGCAACTCTCTACTGTTTCT CCATAGTAGCATAACTTCGTATAGCATACATTATACGAAGTTAT	loxP Data
DSD-4[α]	pBZ23	pBAC- LacZ (F'/ori <sup>V</sup> )	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTGATGCTCG ATGAGGAGAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAAGCTCGAATCCAG TACTCGAGCTCTCTAGGGCGGCGGATTTGTCTACTCAGGAGAGCGTTACCG GACAAACACAGATAAAACGAAAGGCCAGCTTTTCGACTGAGCCTTTTCGTTT TATTTGATGCTCTAGCAGCGGTACCTGGTGGCGCGCCTTATTTGTATAGTTC	loxP FRT Data1

		origins and Cam <sup>R</sup> )	ATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGT GGTCTCTCTTTTCGTTGGGATCTTTTCGAAAGGGCAGATTGTGTGGACAGGTA ATGGTTGTCTGGTAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTGAT AATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGT TAACTTTGATTCCATTCTTTGTTTGTCTGCCATGATGATACATTTGTGTGAGT TATAGTTGATTCCAATTTGTGTCCAAGAATGTTCCATCTCTTTAAATCAAT ACCTTTTAACTCGATTCTATTAAACAGGGTATCACCTTCAAACCTTGACTTCAGC ACGTGTCTTTGAGTTCCCGTCATCTTTGAAAAATAGTTCTTTCCGTGACATA ACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTATATGATCTGGGT ATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTGGCCA TGGAACAGGTAGTTTCCAGTAGTGCAATAAATTTAAGGGTAAGTTTCCGT ATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAAATTTGTGCCATTAACA TCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTAAAAAGTTCTTCTCC TTTACGCATGGTATATCTCTCTTAAAGTGGTCAGTGGCTCCTGCTGATGTG CTCAGTATCTTGTATCCGCTCACAATGTAAATTTGTTATCCGCTCACAATTGTA TCCGCTCATGAATTAATCTTGAAGTCTCTATCTTCTAGAGAATAGGAAC TCAGGCATTGATGAATCGTAGTCTCAATAACTTCGTATAGCATACATTATAC GAAGTTAT	Data2 Data3
SpyTag-Bla	pBZ51	pET28a (pBR322 origin and Kan <sup>R</sup> only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA CATCAGCAGGAGCGCACTGACCACTTTAAGAAGGAGATATACCATGGCCACACA TCGTGATGGTGGACGCTACAAGCCGACGAAGGGTTACAGGGGGTCCGGTCTC ACCCAGAAACGCTGGTGAAGATAAAGATGCTGAAGATCAGTTGGGTGCACG AGTGGGTACATCGAACTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTT CGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGG CGCGGTATTTATCCGCTATTGACGCCGGGCAAGAGCAACTCGGTTCGCCGAT ACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATC TTACGGATGGCATGACAGTAAGAGAAATTATGACGTGCTGCCATAACCATGAG TGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG CTAACCGCTTTTTCGACAACATGGGGATCATGTAACCTCGCTTGATCGTTG GGAACCGGAGCTGAATGAAGCCATACCAACGACGAGCGTGACACCAGAT GCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCAACTTCT ACTCTAGCTTCCCGCAACAATTAATAGACTGGATGGAGCGGATAAAGTTG CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGACGACTGGGGCC AGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCA ACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCTCACTGATTA AGCATTGGTGATAAGTCGACAACCTAGGAAAAACCTGAGGAAAAATGCATAAGC TAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCCTTCGTGTT TTATCTGTTGTTTGTGCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG AGCGGATTTGAACGCTCGCAAGCAACGGCCCGGAGGGTGGCGGGCAGGAC GCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCTGACGGA TGGCCTTTTTCGCTTTCTACAAA	P <sub>LtetO-1</sub> SpyTag Linker Bla Terminator
YcbK- SpyCatcher	pBZ52	pET28a (pBR322 origin and Kan <sup>R</sup> only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA CATCAGCAGGAGCGCACTGACCACTTTAAGAAGGAGATATACCATGGATAAAT TGATGCGAACCCGCGCAAACTGCTGGCGCTGGCGCGCTGGCGCTGGCGG CGCGGATTCTGCGGACCCCGCGCTTTCGACCCCTGAGCACCCCGCGCGAA GCGGTAGTGGAAGTATGGGAGTTGATACCTTATCAGTTTATCAAGTGAGCA AGGTGAGTCCGGTGATATGACAATTGAAGAAGATAGTGCTACCCATATTAAT TCTCAAAACGTGATGAGGACGCGCAAGAGTTAGCTGGTGCAACTATGAGTT GCGTGATTCTCTGTTAAATATTAGTACATGGATTTGAGATGGACAAGTGA AAGATTCTACCTGTATCCAGGAAAAATATACATTTGTGCAAAACCGCAGCACA GACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAATGAGCAAGGTCA GGTTACTGTAAATGGCAAGCAACTAAAGGTGACGCTCATATTTAATGAAG TCGACAACCTAGGAAAAACCTGAGGAAAAATGCATAAGCTAGAGGCATCAAATA AAACGAAAGGCTCAGTCGAAAGACTGGGCCCTTCGTGTTTATCTGTTGTTGTC GGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACCG TGCGAAGCAACGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTG CCAGGCATCAAATTAAGCAGAAGGCCATCTGACGGATGGCCCTTTTTCGCTT TCTACAAA	P <sub>LtetO-1</sub> YcbK SpyCatcher Terminator
MIT Message 1	pBZ63	pET28a (pBR322 origin and Kan <sup>R</sup> only)	GACATTAACTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACTCG AGCTGGTGGCGCGCCTTATTTGTATAGTGCCACGATCCATGCTAACGTCTC TGCGTAGGGATGAATCCCGCTTTTGAACCTGTTTCTACTGACGGACGAGCTGA TAGGTAGCCGAAGTAGTGATACGATCCACACATGCCATCATTGCATACTCGT GCATTCAATGATGATGACATGACGTAGTCCATATGGTAATGGTATGTCAGATC ACATGTCAATACTCGTCACTAGAAGTACGCGCATGACTGGCGAGCTGGTGC GCTCCCGAGGCTGGTGCAGCGACTAAGTTGAATGCGCAGACCGATCGAGAC GACTCTAGCGCTGGAATAAATCAGAATAAAGACCTAGGGATATATTCCGGTTC GCATGTTATCATCAGTAACCCGTATCGTGAGCATCTCTCTCGTTTCATCGG TATCATTACCCCATGAACAGAAATCCCCCTTACACGG	Forward primer MIT message 1 Reverse primer
MIT Message 2	pBZ64	pET28a (pBR322 origin and Kan <sup>R</sup> only)	GACATTAACTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACTCG AGCTGGTGGCGCGCCTTATTTGTATAGCCCACTAATGCTCAATAGACGGT ACTGTACACCCGTGTTTACAGCAACGGGAAAGGAGGATCACTTTCTACAATTG TGTGCTGGAAGTACAGTGCATATCCACACATGCCATCATTGTCATACTCGT CATTCATGATGATCTACAGTAGTCCATATGGTAATGGTATGTCACATACA CATGTCAATACTCGTCACTAGAAGTACGCGCATACGACTCGCCCATAGGGT TCGCCGGCTCGCACTGACTACCTTACGCTCTGACCCAGATCGGAGCCGGCC GCATGACCCCTGTGATATAATACCGTTTCATCCCTAGGGATATATTCCGCTTC CATGTTATCATCAGTAACCCGTATCGTGAGCATCTCTCTCGTTTCATCGGT ATCATTACCCCATGAACAGAAATCCCCCTTACACGG	Forward primer MIT message 2 Reverse primer

**Supplementary Table 5** Identity, plasmid, and sequence information of all constructs used in this study.